Isolation and Genomic Analysis of Bacteria that drive biogeochemical cycling in Prairie Pothole Lake sediments

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Introduction and Background

- Prairie pothole lakes (PPLs) are small wetlands in the upper Midwest/Northern Great Plains of the United States, and southern Canada that cover approximately 750,000 km^2.
- Biogeochemistry of the PPLs is characterized by reducing conditions coupled with high concentrations of dissolved organic carbon (DOC) and varying sulfur species, including sulfate, sulfur, sulfides, and polysulfides.
- Lake sediments and waters contribute to the global cycles of carbon and sulfur by releasing large amounts of greenhouse gasses including methane and carbon dioxide, resulting from the microbial breakdown of complex carbon sources.
- Sulfate reducers are energetically favored in the competition for substrates with microorganisms involved in the methanogenic degradation pathways, resulting in a considerable diversion of the carbon flow from methane to carbon dioxide.
- Sulfide is a toxic compound not typically found excessively in the environment.
- Measurements of sulfide in PPL sediments and pore waters are frequently recorded between 2-3 mM.

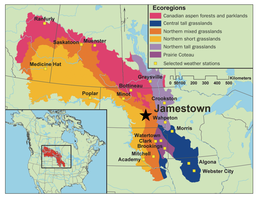


Figure 1. Prairie Pothole Lake Region

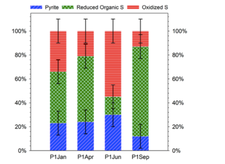


Figure 2. Composition of different sulfur species in lake P1 sediment samples across the four seasons (Zeng et al. 2013)

Experimental Aims

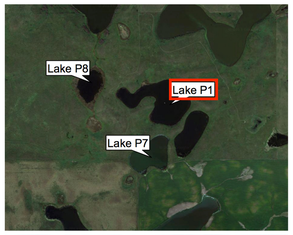


Figure 3. PPL lakes near Jamestown, North Dakota

- Successfully enrich and isolate sulfate reducing bacterium (SRB) and other key environmental microbes
- Explore the characteristics of isolates through physiological analyses
- Utilize genomic data to give insight to SRB metabolism and function in the environment
- Identify any existing relationships with other key players in the community and hypothesize about larger scale regional biogeochemistry

By understanding microbial processes in lake sediments that drive carbon and sulfur cycles, we can further begin to comprehend the role and impact of freshwater wetland systems on the global carbon cycle and apply our knowledge of PPL's to similar systems in other parts of the world.

Isolation Methods

An anaerobic enrichment of PPL sediment from lake P1 at a depth of 16-20cm was initiated October 3rd, 2014. Contents of this enrichment include:

- Freshwater media (FW)
- 20mM sodium sulfate
- 1mL DOC extracted from lake water

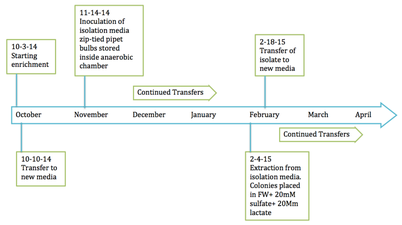


Figure 4. Upper: Isolation media inoculated with enrichment culture
Lower: Turbid isolation media
Right: Anaerobic Chamber. 2-3% Hydrogen gas balanced with Nitrogen gas

Identification of Isolates

- Two isolates resulted from enrichments including a SRB (denoted 1L) and a fermenter, YPD1. 16S rRNA was extracted and confirmed 1L a close neighbor to *Desulfovibrio magneticus*. Genomic DNA for YPD1 was sent for sequencing to the Joint Genome Institute of the United States Department of Energy (JGI) and established its identity as a strain of *Proteiniclasticum ruminis*.
- In February 2016, genomic DNA was extracted from 1L and sent for sequencing to the Genomics Shared Resource (GSR) facility at the Ohio State University using the Illumina HiSeq2500 sequencing platform. A genome with an estimated 3900 genes resulted.
- A maximum likelihood tree was constructed with neighbors from the SILVA and NCBI: BLAST databases for isolate 1L.

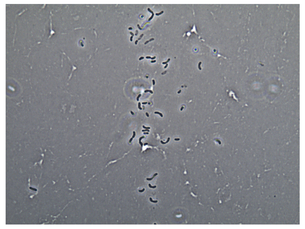


Figure 5. 1L Bright field 1000X

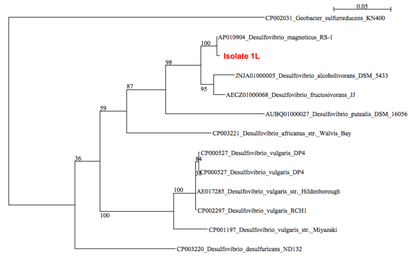
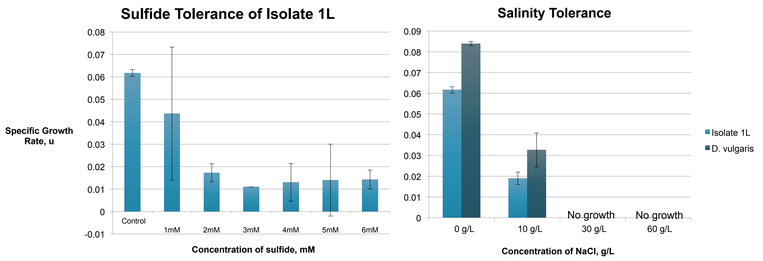


Figure 6. Maximum Likelihood phylogenetic tree

Physiology and Characterization

The physiology of 1L was characterized by experiments for sulfide tolerance, salinity tolerance, and growth on various organic and inorganic substrates.



- Preliminary data suggested sulfur species were tolerating ~2mM sulfide in their natural environment. Knowing this, cultures were spiked at the time of inoculation with sulfide concentrations ranging from 1mM – 6mM.
- Growth was measured via optical density at 600nm
- Cultures of 1L were placed under various concentrations of sodium chloride ranging from 0 to 130g/L
- Growth was measured via optical density at 600nm and readings were taken every 1-2 hours accordingly over a span of 314 hours.

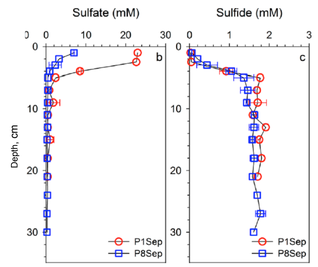


Figure 7. Zeng, ES&T, 2013

Substrate Utilization

Electron Acceptor	Electron Donor	Growth?
Sulfate	Acetate	**Minimal/No**
Sulfate	Butyrate	**No**
Sulfate	Formate	**No**
Sulfate	Propionate	**No**
Sulfate	lactate	**Yes**
Sulfate	Ethanol	**Yes**
Thiosulfate	lactate	**Yes**

The sodium salts of each substrate (excluding ethanol) were used to amend cultures at a concentration of 20mM. Growth was assessed by comparing OD600 readings to a standard 1L culture on sulfate and lactate in freshwater media.

1L: Genomic Preview

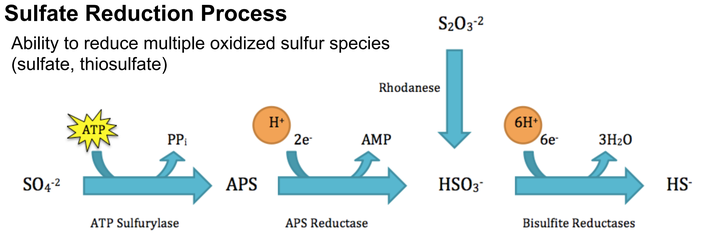
The draft genome of isolate 1L was annotated and resulted in a predicted ~3900 genes across 233 scaffolds. The following are genes of interest currently identified. Further analysis via the program ITEP (An integrated toolkit for exploration of microbial pan-genomes) will be used to observe genes conserved across related organisms.

Genes
Carbon monoxide dehydrogenase
Ech hydrogenase subunits A to F
Dissimilatory sulfite reductase (α, β, γ)
Sulfate adenylyltransferase
Adenylylsulfate reductase
Rhodanese
Nif genes for nitrogen fixation
Putative Phage sequence
CRISPR elements (cas1, 2, 3, 4, 5, csd2)
Methyl-Accepting chemotaxis proteins

ITEP Statistics	
Total genes among 11 organisms	14,758
Total genes in common	856
Genes Unique to 1L	336
1L unique genes with unknown function	109

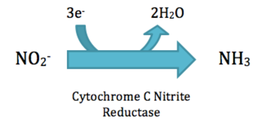
Sulfate Reduction Process

Ability to reduce multiple oxidized sulfur species (sulfate, thiosulfate)



DNRA (dissimilatory nitrate reduction to ammonia)

Mechanism for disposing of excess reductant in environments with abundant electron donor



Direction and Discussion

We successfully isolated a sulfate-reducing bacterium and a fermenter under anaerobic conditions. Physiological experimentation has helped us understand 1L's metabolic capabilities, with this isolate showing some tolerance to elevated aqueous sulfide concentrations. Further analysis of this microorganism's genome will enable further hypotheses to be developed and tested, and will enable inferences to be made regarding interactions between such SRB and other community members in PPL sediments and pore waters. Such interactions likely play key roles in carbon and sulfur cycling in these environments and also regulating contaminants and compounds.

Future Research Goals

- Expand our knowledge of isolate 1L by exploring its genome
- Derive a relationship between isolate 1L and other community members (methanogenic archaea)
- Use our scaled system to test our hypotheses about larger scale biogeochemistry in the Prairie Pothole Lake region